Assessment of Heat-Damaged Wheat Kernels Using Near-Infrared Spectroscopy[1]

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ABSTRACT
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Heat damage is a serious problem frequently associated with wet harvests because of improper storage of damp grain or artificial drying of moist grain at high temperatures. Heat damage causes protein denaturation and reduces processing quality. The current visual method for assessing heat damage is subjective and based on color change. Denatured protein related to heat damage does not always cause a color change in kernels. The objective of this research was to evaluate the use of near-infrared (NIR) reflectance spectroscopy to identify heat-damaged wheat kernels. A diode-array NIR spectrometer, which measured reflectance spectra (log (1/R)) from 400 to 1,700 nm, was used to differentiate single kernels of heat-damaged and undamaged wheats. Results showed that light scatter-

ing was the major contributor to the spectral characteristics of heat-damaged kernels. For partial least squares (PLS) models, the NIR wavelength region of 750–1,700 nm provided the highest classification accuracy (100%) for both cross-validation of the calibration sample set and prediction of the test sample set. The visible wavelength region (400–750 nm) gave the lowest classification accuracy. For two-wavelength models, the average of correct classification for the classification sample set was >97%. The average of correct classification for the test sample set was generally >96% using two-wavelength models. Although the classification accuracies of two-wavelength models were lower than those of the PLS models, they may meet the requirements for industry and grain inspection applications.

Heat damage is an important grading factor for wheat in the United States. Heat damage can be caused by excessive heating from artificial drying, or spontaneous heating during storage and can injure gluten quality of wheat (Halverson and Zeleny 1988). Heat-damaged kernels account for ≈10–25% of the total damaged kernels from U.S. grade Nos. 1 to 5 (USDA 1988). They are defined as kernels and pieces of kernels that are materially discolored and damaged by heat. Heat damage results in protein denaturation and reduces gluten quality. In some cases, even if the temperature is not high enough to cause discoloration, gluten quality may be impaired (Halverson and Zeleny 1988). Use of heat-damaged wheat can affect processing, resulting in changes in physical properties of dough and loss of baking quality (Becker and Sallans 1956; McDermott 1971; Hook 1980; Preston et al 1989).

The current inspection method for determining heat-damaged kernels relies on visual examination of wheat kernels and, in some cases, a cross-sectional analysis to determine whether the color is reddish-brown or mahogany. Results from this form of analysis vary greatly and depend on the inspector's experience. Heat damage due to improper storage usually occurs with a fungal attack, resulting in both heat damage and visual fungal damage. Kernel discoloration caused by fungus infestation on the seed coat is easy to recognize and is the probable reason that heat damage from bin heating can be accurately determined visually. However, denatured protein related to heat damage does not always cause a change in kernel color. Therefore, some heat-damaged kernels cannot be found with visual examination.

Several other methods to assess heat damage in wheat have been studied. A mixograph has been routinely used for heat-damage determination (Kilborn and Tipples 1981; Preston et al 1989; Preston and Symons 1993). Measurements of changes in physical dough properties and baking quality provide the most meaningful tests of heat damage because they relate directly to processing quality. Unfortunately, these tests are time-consuming and require expensive

equipment and a high degree of technical expertise. Researchers have determined heat damage by enzyme inactivation, dye binding capacity, turbidity, and protein extractability (McDermott 1971; Hook 1980; Every 1987). All four of those methods are based on the effects of heat on grain protein denaturation and are fairly complicated procedures. Seckinger and Wolf (1970) and Preston and Symons (1993) studied the measurement of heat damage in wheat by assessment of protein fibril formation. A small amount of endosperm material obtained from a milling or grinding sample was placed on a microscope slide, wet with a drop of water, and observed. They found that heat damage reduced the protein fibril formation. This procedure requires technical training when reference photographs are used. Thus, the available methods for assessing heat damage are either time-consuming or complicated. An objective method that is rapid, precise, nondestructive, and based on single kernels would benefit both the food industry and the U.S. Department of Agriculture Grain Inspection, Packers, and Stockyards Administration (GIPSA).

Near-infrared (NIR) reflectance spectroscopy is a method that may meet those requirements. NIR can be used to measure both physical and chemical properties of agricultural products, by-products, and food. Grain drying, especially high-temperature drying (>60°C), can cause stress cracks, irreversible protein denaturation, and starch gelatinization (Myers 1990; Brooker et al 1992). The stress cracks in the kernel could result in light scattering that, in turn, affects the energy absorption pattern. Irreversible protein denaturation changes the native folding of protein to an aggregate and forms an unfolding rigid entity. The transition from native protein to denatured protein has two stages, aggregation and cross-linking. Protein aggregation involves the formation of a higher molecular weight complex, and cross-linking forms specific bonding at specific sites on the protein strands (Wall et al 1975; Wight 1981; Myers 1990). Those chemical structure changes may also result in NIR absorption differences between native protein and denatured protein. Thus, classifying heat-damaged wheat and undamaged wheat by NIR reflectance spectroscopy might be possible.

Recent research on single wheat kernel quality measurement using NIR spectroscopy has successfully measured protein content (Delwiche 1995, 1998); wheat class (Song et al 1995; Delwiche and Massie 1996); color class (Dowell 1997, 1998; Wang et al 1999a–c); and insect damage (Dowell et al 1998; Baker et al 1999; Ridgway et al 1999). However, the use of NIR spectroscopy to measure kernel heat damage, especially in combination with other quality measurements, has not been studied. Therefore, the objective of this research was to evaluate the use of NIR reflectance spectroscopy to identify heat-damaged wheat kernels.

MATERIALS AND METHODS

Sample Preparation

Hard red spring wheat (HRS) and hard white wheat (HWW), obtained from GIPSA (Kansas City, MO), were used. Heat-damaged wheat samples were created by drying undamaged wheat (Preston and Symons 1993). Before drying, wheat samples were tempered to 17% moisture content in sealed containers for at least two days at 4°C. Then ≈300 g of each wheat class was sealed in a 500-mL Pyrex-plus media bottle (Fisher Scientific Co., Pittsburgh, PA) and placed in a forced-air oven for 16 hr at 70°C. The bottle with a one-piece teal polypropylene plug-seal cap is autoclavable up to 121°C. Therefore, there was no moisture loss from the bottle during heating. After heating, wheat samples were removed from the bottle and conditioned for at least 10 days at room temperature before spectra were viewed. Wheat samples without heat treatment were used as the control. Chemical compositions of the samples used are shown in Table I.

Kernel Color Measurement

Reflectance spectra at 480–750 nm were transferred into $L*a*b*$ color space. Grams/32 (Galactic Industries, Salem, NH) software was used for kernel color determination. In the $L*a*b*$ color space, $L*$ varies from 0 (black) to 100 (perfect white); $a*$ varies from –100 to 100 and measures green when negative and red when positive; $b*$ varies from –100 to 100 and, is a measure of blue when negative and yellow when positive.

Mixograph Assessment of Heat Damage

A mixogram was used to determine heat damage to wheat. Mixograph curves were obtained using standard laboratory procedures (Approved Method 54-40A, AACC 2000). Flour (10 g) was mixed with the optimum amount of water for 8 min, or until mix time could be determined, at 26°C in a mixograph bowl. A mixograph pattern was analyzed to provide mixing requirement, mixing tolerance, and optimum water absorption. Wheat flour used for obtaining mixograph curves was prepared using a laboratory mill (60–65% extraction).

RVA Assessment of Heat Damage

A Rapid Visco Analyser (RVA) model 3-CR (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was used. Sample preparation and testing were based on Approved Method 76-21 (AACC 2000). Gelatinization, pasting, and setback profiles were measured at a ramping rate of 12.2°C/min in 13 min. The temperature profile was divided into three sections, low temperature, high temperature, and cooling-setback. The temperature profile started at 50°C and was held for about 1 min, then the temperature started to increase. In ≈4 min, it reached 95°C and was held at this temperature for 3 min. After ≈4 min of cooling, the temperature decreased to 50°C and was held at this temperature for ≈1 min.

NIR Spectra Collection

A diode-array NIR spectrometer (DA7000, Perten Instruments, Springfield, IL) was used to quantify single kernels of heat-damaged and undamaged wheats. The diode-array spectrometer measures visible (400–750 nm) and NIR (750–1,700 nm) reflectance at a rate of 30 spectra/sec. Single wheat kernels were placed in a black V-shaped trough (12 mm long, 10 mm wide, and 5 mm deep) and illuminated with white light from a fiber bundle (8 mm diameter) positioned 13 mm from the top of the trough and oriented 45° from vertical. The reflectance probe (2 mm diameter) was oriented vertically 9.5 mm from the top of the trough. The reflectance probe carried the reflected energy to a spectrometer. The procedures included collecting a baseline, collecting eight spectra from each of the kernels, and averaging the eight spectra for each kernel. A total of 260 heat-damaged and 260 undamaged kernels from HRS and HWW were measured, for a total of 1,040 kernels. A spectrum of the empty trough was measured as a reference before kernel measurement and again after every 100 kernels.

Statistical Analysis and Model Development

Partial least squares (PLS) (Galactic Industries, Salem, NH), used for data analysis and modeling is a multivariate data analysis technique designed to handle intercorrelated regressors. Two-class models were developed to classify heat-damaged and undamaged kernels. Wheat kernels first were separated equally into calibration and testing sets, based on even and odd numbers. Heat-damaged kernels and undamaged kernels were assigned constant values of 1.0 and 2.0, respectively. A kernel was considered to be correctly categorized if the predicted value lay on the same side of the midpoint of assigned values. The model performance is reported as the cross-validation of each calibration sample set and prediction of test sample sets. The number of PLS factors was the minimum required to give the best classification results. Stepwise discriminant (STEPDISC) analysis was used to develop two-wavelength models for classification of heat-damaged and undamaged wheat kernels. STEPDISC uses forward selection and backward elimination to select a subset of wavelengths for classifying each spectrum into classes. The wavelengths are chosen to enter or leave the model according to the significant level of an F-test from an analysis of covariance and the model with highest classification accuracy.
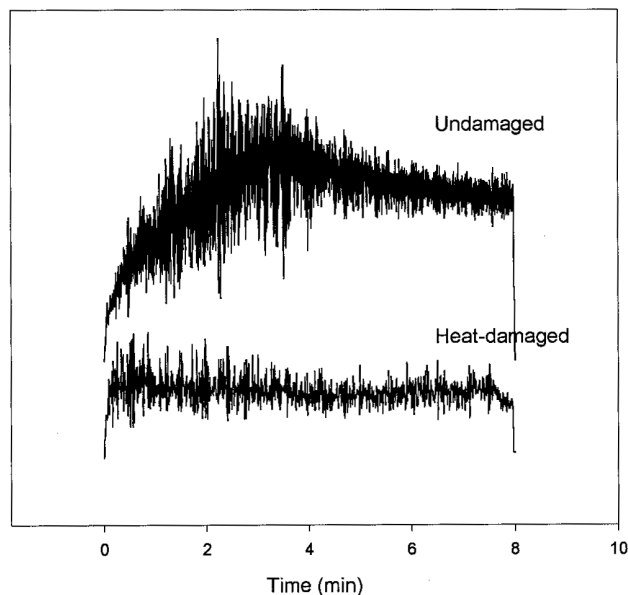

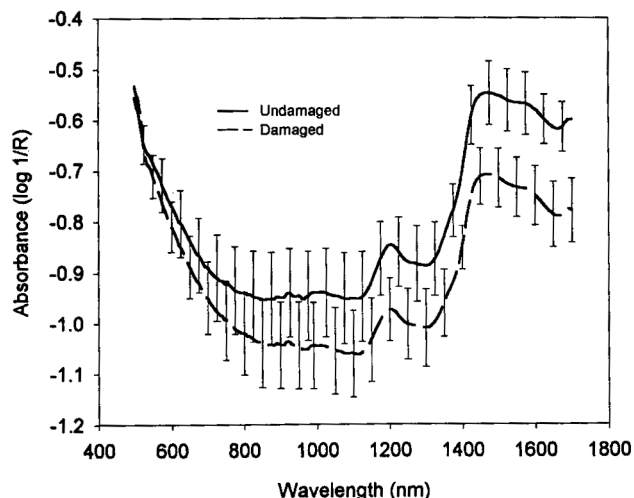**Fig. 1.** Mixograph curves for heat-damaged and undamaged wheat.

Fig. 2. NIR absorption curves for heat-damaged and undamaged wheat. Vertical bar represents one standard deviation.

RESULTS AND DISCUSSION

Mixograph and RVA Assessments

The mixograph curve of undamaged wheat showed a strong and high peak at 180 sec after mixing (Fig. 1). The mixograph curve of heat-damaged wheat showed a flat line through the mixing period. This indicates that protein in wheat was denatured, and dough lost baking quality (Becker and Sallans 1956; McDermott 1971; Hook 1980; Preston et al 1989). Disulfide bonds of the gluten play an important role in determining gluten and dough properties (Lásztity 1996). Dough structure is based on an extensive three-dimensional bonding of the protein subunits joined together by disulfide cross-links, and heat-damage breaks these bonds.

RVA curves showed that heat-damaged wheat had a higher peak viscosity and lower final viscosity than undamaged wheat. Also, the heat-damaged wheat reached peak viscosity earlier than undamaged wheat. This probably was due to starch damage, resulting in easier water absorbing and faster swelling than undamaged starch. However, no significant difference was found between damaged and undamaged wheat. Therefore, detailed results from the RVA test are not reported.

PLS Model Assessment

Classification results of cross-validation of calibration sample sets and prediction of test sample sets using PLS models with seven PLS factors are summarized in Table II. The NIR wavelength region of 750–1,700 nm gave the highest percentage of correct classification for both cross-validation and prediction (100%). The visible wavelength region gave the lowest percentage of classification. Figure 2 shows the average spectra of heat-damaged and undamaged wheat kernels. In general, the log (1/R) values of heat-damaged kernels were lower. Differences in log (1/R) value between heat-damaged and undamaged kernels increased as wavelength increased. In addition, differences in log (1/R) value between heat-damaged and undamaged kernels were smaller in the visible region than in the NIR region.

Light scattering probably caused the log (1/R) value differences between heat-damaged and undamaged wheat. Grains are frequently physically damaged internally during high-temperature drying (Brooker et al 1992). During high-temperature drying, stress-cracks may originate in the endosperm at the center of the kernels and propagate outward to the aleurone layer along the boundary of the

starch granules. These cracks increase air space inside the kernels. Therefore, heat-damaged kernels have more air spaces that diffract and diffuse light. This results in more incident energy reflected to the NIR sensor, hence, lower absorbance occurs in heat-damaged kernels. Undamaged kernels have no internal cracks or have less air space to diffract light. Thus, light entering undamaged kernels is more likely to pass through the kernel, which results in a higher absorbance measured by the NIR sensor.

Two-Wavelength Model Assessment

Classification results of calibration sample sets and prediction of test sample sets using two-wavelength models are summarized in Table III. The wavelengths used in each model were those that produced the highest R^2 and highest percentage of correct classification for the calibration sample sets. Three pairs of wavelengths in the visible and near-infrared regions were compared. Wavelengths selected from the NIR region yielded better results (96.8%) than wavelengths selected from the visible region (94.3%). An instrument with one or two wavelengths is less expensive than a dispersive instrument because only a simple filter is required. The lowest classification accuracy of 94.3% from the two-wavelength model may meet the requirements for industry and grain inspection applications. Therefore, the two-wavelength model is more practical.

Difference in color between heat-damaged and undamaged wheat kernels was the major contributor to the classification accuracy of the two-wavelength model using wavelengths of 505 and 560 nm. Kernel color measurement results showed that heat-damaged kernels had higher $a*$ and $b*$ values than undamaged kernels (Table IV). This indicates that heat-damaged kernels are darker and more yellow than undamaged kernels. Heat damage causes comprehensive physical and chemical changes in wheat. The wavelengths for the other two models may represent scattering effects and interactions of moisture, starch, protein, oil, and cellulose caused by heat-damage.

TABLE IV
Color Variations Between Heat-Damaged and Undamaged Wheat Kernels Measured as *L,a,b* Values

Class	L	a	b
Hard red spring wheat			
Heat-damaged	61.14a[a]	16.02a	25.09a
Undamaged	60.61a	12.96b	16.15b
Hard white wheat			
Heat-damaged	71.33a	14.96a	23.34a
Undamaged	68.53b	12.14b	19.07b
All samples			
Heat-damaged	66.24a	15.91a	24.21a
Undamaged	64.56a	12.54b	17.61b

[a] Values within the same column followed by different letters are significantly different at $P<0.05$.

TABLE I
Composition of Wheat Samples (% db)

Wheat Class[a]	Protein	Starch	Ash	Fiber
HWW	15.88	64.9	1.66	2.05
HRS	19.89	58.5	1.75	1.32

[a] Hard white wheat (HWW) and hard red spring wheat (HRS).

TABLE II
Summary of Cross-Validation and Testing Results Using Partial Least Squares (PLS) Models for Classification of Heat-Damaged and Undamaged Wheat Kernels

Wavelength (nm)	n	Cross-Validation (%)			Testing Results (%)		
		Undamaged	Damaged	Average	Undamaged	Damaged	Average
400–750	520	99.2	99.2	99.2	98.8	98.1	98.4
400–1,700	520	99.2	99.6	99.4	98.8	98.4	98.6
750–1,700	520	100	100	100	100	100	100

TABLE III
Classification of Heat-Damaged and Undamaged Wheat kernels Using Two-Wavelength Models

Model	Wavelength (nm)	Calibration Results (%)			Testing Results (%)		
		Undamaged	Damaged	Average	Undamaged	Damaged	Average
1	505, 560	98.1	96.5	97.3	96.8	91.8	94.3
2	985, 1,050	97.0	98.1	97.5	96.1	97.4	96.8
3	1,550, 1,575	96.2	98.3	97.3	94.1	99.3	96.7

CONCLUSIONS

The log (1/R) values of undamaged wheat kernels were higher than those of heat-damaged wheat kernels over the entire wavelength region. The main contributors to the spectral characteristics of heat-damaged kernels are light scattering and color that changes the log (1/R) values of the absorption spectrum. For PLS models, classification accuracy of 100% was obtained for both cross-validation and prediction from the NIR wavelength region of 750–1,700 nm. The visible wavelength region (400–750 nm) gave the lowest classification accuracy. For two-wavelength models, the average of correct classification for the calibration sample set was >97%. The average of correct classification for the test sample set was ≈96%. Although the classification accuracies of two-wavelength models were lower than those of the PLS models, the classification accuracy of ≈97% may meet requirements for industry and grain inspection applications. Therefore, the simple two-wavelength model is recommended.

Results from this limited sample set and heat treatment suggest that NIR technology could be used to detect heat-damage in wheat. More research is needed with a larger number of samples and a wider range of heat treatments to confirm these results.

LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Methods 54-40A and 76-21. The Association: St. Paul, MN.

Baker, J. E., Dowell, F. E., and Throne, J. E. 1999. Detection of parasitized rice weevils in wheat kernels with near-infrared spectroscopy. Biol. Control 16:88-90.

Becker, H. A., and Sallans, H. R. 1956. A study of the relationship between time, temperature, moisture content, and loaf volume by the bromate formula in the heat treatment of wheat and flour. Cereal Chem. 33:254-265.

Brooker, D. B., Bakker, F. W., and Hall, C. W. 1992. Drying and Storage of Grains and Oilseeds. D. B. Brooker, F. W. Bakker, and C. W. Hall eds. Van Nostrand Reinhold: New York.

Delwiche, S. R. 1995. Single wheat kernel analysis by near-infrared transmittance: Protein content. Cereal Chem. 72:11-16.

Delwiche, S. R. 1998. Protein content of single kernels of wheat by near-infrared reflectance spectroscopy. J. Cereal Sci. 27:241-254.

Delwiche, S. R. and Massie, D. R. 1996. Classification of wheat by visible and near-infrared reflectance from single kernels. Cereal Chem. 73:399-405.

Dowell, F. E. 1997. Effect of NaOH on visible wavelength spectra of single wheat kernels and color classification efficiency. Cereal Chem. 74:617-620.

Dowell, F. E. 1998. Automated color classification of single wheat kernels using visible and near-infrared reflectance. Cereal Chem. 75:142-144.

Dowell, F. E., Throne, J. E., and Baker, J. E. 1998. Automated nondestructive detection of internal insects infestation of wheat kernels using near-infrared reflectance spectroscopy. J. Econ. Entomol. 91:899-904.

Every, D. J. 1987. A simple, four-minute, protein-solubility test for heat damage in wheat. J. Cereal Sci. 6:225-236.

Halverson, J., and Zeleny, L. 1988. Criteria of wheat quality. Pages 15-45 in: Wheat Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Hook, S. C. W. 1980. Dye-binding capacity as a sensitive index for the thermal denaturation of wheat protein. A test for heat-damaged wheat. J. Sci. Food Agric. 31:67-81.

Kilborn, R. H. and Tipples, K. H. 1981. Canadian test baking procedures I. GRL Remix method and variations. Cereal Foods World 26:624.

Lásztity, R., ed. 1996. Wheat protein. Pages 19-138 in: The Chemistry of Cereal Proteins. CRC Press: Boca Raton, FL.

McDermott, E. E. 1971. The 'turbidity' test as a measurement of thermal denaturation of protein in wheat. J. Sci. Food Agric. 22:69-72.

Myers, C. D. 1990. Study of thermodynamics and kinetics of protein stability by thermal analysis. Pages 16-50 in: Thermal Analysis of Foods. V. R. Harwalker and C. Y. Ma, eds. Elsevier Applied Science: London.

Preston, K. R., Morgan, B. C., Kilborn, R. H., and Tipples, K. H. 1989. Assessment of heat damage in Canadian hard red spring wheats. Can. Inst. Food Sci. Technol. J. 22:63-69.

Preston, K. R., and Symons, S. J. 1993. Measurement of heat damage in wheat by assessment of protein fibril formation. J. Cereal Sci. 18:53-59.

Ridgway, C., Chambers, J., and Cowe, I. A. 1999. Detection of grain weevils inside single wheat kernels by a very near infrared two-wavelength model. J. Near Infrared Spectrosc. 7:213-221.

Seckinger, H. L., and Wolf, M. J. 1970. Electron microscopy of endosperm protein from hard and soft heats. Cereal Chem. 47:236-243.

Song, H., Delwiche, S. R., and Chen, Y. R. 1995. Neural network classification of wheat using single kernel near-infrared transmittance spectra. Optical Eng. 34:2927-2934.

USDA. 1988. Wheat. Chapter 12 in: Grain Inspection Handbook. USDA-FGIS: Washington, DC.

Wall, J. S., James, M. J., and Donaldson, G. L. 1975. Corn protein: Chemical and physical changes during drying of grain. Cereal Chem. 52:779-790.

Wang, D., Dowell, F. E., and Lacey, R. E. 1999a. Single wheat kernel color classification using neural networks. Trans. ASAE 42:233-240.

Wang, D., Dowell, F. E., and Lacey, R. E. 1999b. Single wheat kernel size effects on reflectance spectra and kernel color classification. Cereal Chem. 76:34-37.

Wang, D., Dowell, F. E., and Lacey, R. E. 1999c. Single wheat kernel color classification using near-infrared reflectance spectra. Cereal Chem. 76:30-33.

Wight, A. W. 1981. Changes in properties of some maize cultivars associated with artificial drying at elevated temperature. II. Protein solubility and other properties in relation to milling quality. Starch 33:165-168.